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GASTROINTESTINAL ENDOSCOPY

Endoscopic placement of collagen at the lower esophageal sphincter to inhibit gastroesophageal reflux: a pilot study of 10 medically intractable patients

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Ten highly symptomatic and medically refractory refluxing patients were treated with a new endoscopic technique to decrease gastroesophageal reflux. Cross-linked bovine dermal collagen was injected beneath the mucosa in the area of the lower esophageal sphincter through a 23 gauge needle-tipped catheter. A mean volume of 85 ml of implant was injected in 0.5- to 4-ml increments over 3 to 10 injection sessions. All patients developed objective evidence of decreased reflux by one or more parameters. Nine out of 10 patients had decreased symptoms, and 8 of 9 patients had an increase in lower esophageal pressure after implant injection. Endoscopic implant treatment resulted in statistically significant improvement in symptom scores ($p < 0.001$), the standard acid reflux test ($p = 0.009$), and lower esophageal sphincter pressures ($p = 0.002$), but not in the endoscopic appearance of the esophagus ($p = 0.131$). Subjective and objective improvements in reflux parameters generally lasted 6 to 9 months with return toward pretreatment status by 12 months. Antibodies to bovine collagen developed in 5 of 10 subjects with no clinical sequelae and no apparent reactivity with human collagen. The technique is not difficult to perform and is well tolerated by patients, and the results indicate the potential for more general use with a more suitable implant material. (Gastrointest Endosc 1988;34:106–112)

Occasional episodes of gastroesophageal reflux (GER) occur in nearly everyone. Symptomatic reflux is common and medically refractory reflux is not rare. Approximately 80% to 90% of patients whose symptoms are not adequately controlled with relatively simple postural, dietary, and pharmacologic regimens benefit from an antireflux surgical procedure. Unfortunately, patients with poorly controlled symptoms or the complications of reflux may fail to respond to multiple drug regimens, be poor surgical candidates, or fail to achieve sustained benefit from surgery. Effective, nonsurgical treatment for this subset of medically refractory, refluxing patients does not exist. It was hypothesized that the endoscopic injection of a biocompatible material into the submucosa of the distal esophagus to approximate the folds of the terminal rosette could decrease the frequency of GER without disrupting physiologic swallowing mechanisms.

The rationale for the technique and choice of implant material was based on the following premises. First, reflux strictures are commonly associated with resolution of inflammation proximal to the narrowing. Second, endoscopic sclerosis of esophageal varices employing shallow injections into the distal esophagus is generally safe and well tolerated. Third, mechanical support of the distal esophagus, whether by conventional antireflux operations or the Angelchik prosthesis, impedes GER and raises the lower esophageal sphincter (LES) pressure. Additionally, injectable implants are used successfully to support soft tissue in other areas of the body, e.g., the vocal cords and the urinary sphincter.[1–3] Finally, cross-linked bovine der-

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mal collagen has been shown to be a biocompatible material and has a viscosity that allows it to be injected through a long catheter and small gauge needle.

Previous dog experiments[4] have shown the feasibility and efficacy of implanting inert materials into the submucosa of the LES to inhibit GER.

MATERIALS AND METHODS

This study was approved by the Indiana University Investigational Review Board to determine whether this mode of treatment could diminish the subjective and objective features of reflux, how long the effects of treatment would persist, and whether unanticipated side effects of treatment would occur. Patients referred for treatment had been treated with combinations of H_2 blockers, bethanechol, metoclopramide, carafate, and antacids, but they remained severely symptomatic and were willing to accept experimental treatment. Half of the patients had had at least one surgical antireflux procedure, and all had declined surgery prior to enrollment. The criteria for inclusion and exclusion in this study are summarized in Table 1, and the protocol followed is detailed in Table 2. The endpoint of treatment was symptom control, which generally correlated with good approximation of the folds of the terminal rosette of the esophagus.

The endoscopic technique differs little from that of esophageal sclerotherapy. A 23 gauge needle-tipped catheter was inserted tangentially into the submucosa, and the implant was injected within 2 cm of the squamocolumnar junction. The amount of implant injected (0.5 to 4 ml) was determined by the appearance of the submucosal mound. The desired effect was a rounded bulge into the lumen without blanching of the overlying mucosa (Figs. 1 to 3). Glutaraldehyde cross-linked fibrillar collagen was supplied by Collagen Corporation (Palo Alto, Calif.) at 35 mg/ml in phosphate-buffered saline (pH 7.2) with 0.3% lidocaine.

The endoscopic grading of esophagitis was as follows: 0 = normal, 1 = fine vessel prominence and/or localized erythema, 2 = linear erythema, 3 = linear erosions, 4 = ulcer, stricture, or columnar epithelial metaplasia (Barrett's esophagus).

Table 1.
Protocol admission and exclusion criteria

Admission criteria

I. Medically intractable gastroesophageal reflux (disabling symptoms while taking at least 3 antireflux drugs, i.e., an H_2 blocker, sucralfate, metoclopramide, urecholine, frequent antacids)
II. Failed surgical repair or very high surgical risk and either:
  A. Frequent aspiration of gastric contents
  or
  B. Two of the following:
    1. Endoscopic esophagitis (at least as severe as linear erythema)
    2. SART with 3 or more reflux episodes
    3. Reflux by barium esophagram or nuclear scintiscan
    4. Positive acid perfusion test (Bernstein test)
    5. Manometric lower esophageal sphincter pressure of less 10 mm Hg

Exclusion criteria

I. History of autoimmune disease or anaphylaxis
II. Esophageal cancer, varices, or infection

Table 2.
Procedure

1. Baseline studies to establish the presence of reflux (endoscopy, esophagram, SART, Bernstein test, nuclear scintiscan, manometry).
2. Skin test: 0.1 ml of implant material was injected into the dermis of the forearm at least 1 month prior to endoscopic implant placement. (A skin test was interpreted as nonreactive if no erythema, pruritis, or pain developed during the month of observation.)
3. Materials: A 3.5-mm channel endoscope and a 23 gauge needle-tipped catheter. Cross-linked bovine dermal fibrillar collagen was supplied by Collagen Corporation, Palo Alto, California.
4. Technique: Standard endoscopic premedication with the addition of atropine. Injections of 0.5 to 4 ml of implant in 3 to 8 sites at the squamocolumnar junction ±2 cm.
5. Oral analgesics for 1 to 2 days, as needed.
6. Repeat injections at 2- to 4-week intervals until symptoms were controlled and the lower esophageal sphincter appeared and felt competent to the endoscopist, i.e., was no longer patulous.
7. Tapering and discontinuation of antireflux medications as permitted by symptoms.
8. Repetition of baseline studies when implants were completed and at 6 and 12 months.

Standard acid reflux test (SART) data were obtained using an antimony pH sensor (Konigsberg Instruments, Inc., Pasadena, Calif.) positioned 5 cm above the LES. The patient assumed four positions (supine, left and right lateral decubitus, and seated leaning forward) and performed four respiratory maneuvers (Valsalva, Mueller, cough, deep breathing) in each position for 1 min. Consequently, there were 16 potential GER opportunities per test session.

LES manometry was performed with a Sandhill Diagnostic Motility System (Sandhill Scientific, Littleton, Colo.) using a station pull-through technique. LES pressures were recorded from midrespiratory readings using the intragastric pressure as the zero baseline (normal pressures are 13 to 50 mm Hg).

Nuclear scintiscans were performed after at least a 4-hour fast. Technetium-99m (2.4 mCi) was administered orally in 2 to 3 ml of saline followed by 300 ml of acidified orange juice. If free reflux did not occur in the baseline supine state, an abdominal binder with sphygmomanometer was inflated by up to 100 mm Hg. GER was read as either present or absent.

In performing the esophagrams, the radiologist attempted to demonstrate GER by positioning the supine patient head-down 10 to 15 degrees and having the patient perform Valsalva maneuvers.

On a questionnaire administered by a nurse patients rated their symptom severity on a scale of 0 to 4 (0 = asymptomatic, 1 = mild, 2 = moderate, 3 = severe, 4 = intolerable symptoms). The patients also were asked to state whether they were better, worse, or unchanged since their last visit and since beginning treatment. To further confirm and clarify any change in clinical status, the patients were queried at each clinic visit about their symptom frequency, the activities that provoked them, the number of medications and how often they were taken, and which dietary and/or

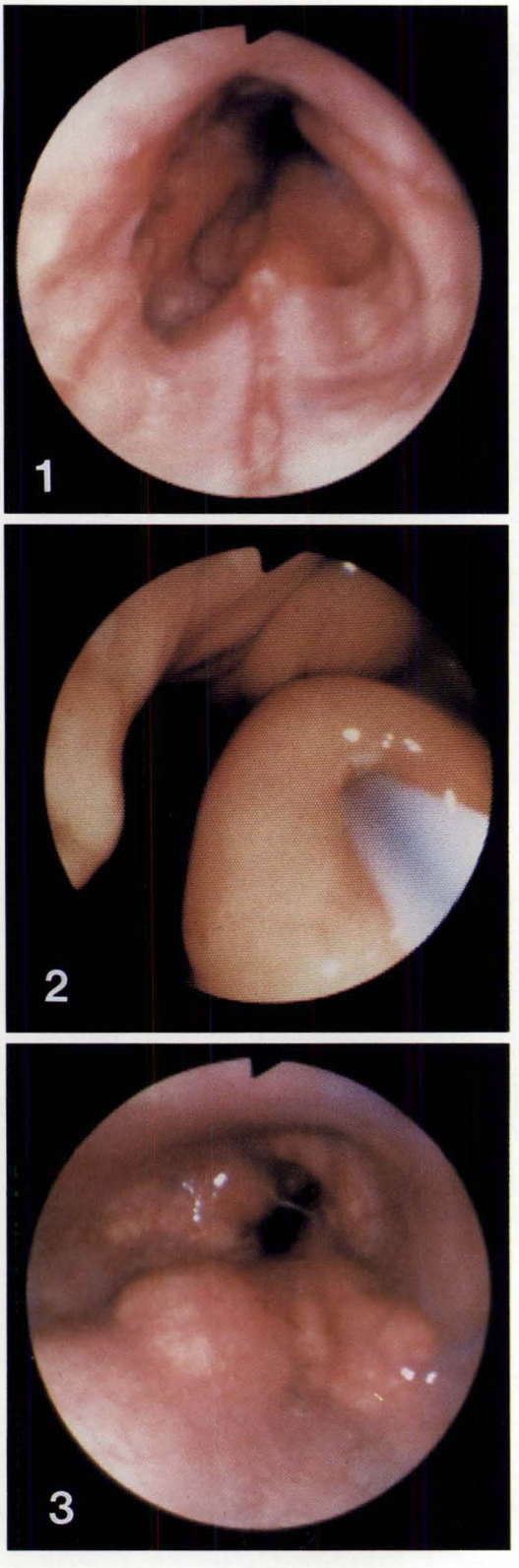

Figure 1. The preinjection appearance of a distal esophagus showing the linear erosions.
Figure 2. Endoscopic injection of the implant beneath the mucosa of the distal esophagus.
Figure 3. The postinjection appearance of the distal esophagus showing the submucosal implants bulging into the lumen.

behavioral adjustments in daily activities were being observed.

Sera to measure antibodies to bovine dermal collagen were drawn before skin-testing and at approximately 6-month intervals. Antibody titers were measured by an enzyme-linked immunosorbent assay (ELISA).

Statistical analysis was performed with SAS (statistical analysis system). A comparison of means was made using analysis of variance. When a significant difference was found among means ($p < 0.05$), a multiple comparison technique (least significant difference) was used to compare individual means.

Because of the irregular appearance both radiographically and endoscopically of the distal esophagus after implant treatment, all protocol patients were given cards to carry in their wallets explaining the effect of the treatment on the appearance of the esophagus and providing the phone numbers of the investigators.

RESULTS

The general characteristics of the 10 patients are listed in Table 3. The patients underwent a mean of 6.3 implant sessions (range, 3 to 10) and received a mean volume (±SD) of 85 ± 34 ml of collagen (range, 27 to 139 ml). Nine subjects completed the 12-month follow-up phase of the study. One subject with nearly continuous reflux withdrew from the study after partial but unacceptable relief of pain and persistent vomiting despite improvement in all objective reflux parameters. A technically successful fundoplication was performed without decreasing her symptoms. This subject unfortunately had two sources of epigastric pain that she could not distinguish and ultimately achieved pain relief from a sphincteroplasty of the sphincter of Oddi performed for papillary stenosis. SART and manometric data were not obtained for the patient with an esophagojejunostomy as the patient was achlorhydric and the LES was surgically absent.

Symptom severity was scored on a 0 to 4 scale by each patient before treatment, at the conclusion of implantation, and 6 and 12 months after the last implant injection (Fig. 4). The mean pretreatment symptom score was 3.2, immediately after treatment was complete it was 1.3, 6 months later it was 1.6, and 1 year after treatment it was 1.7. The pretreatment

Table 3.
Patient characteristics

	No.
Mean age (yr)	46 ± 22
Female:male	9:1
Medical failure	10:10
Surgical failure	5:10
Reoperation failure	3:10
Morbidly obese	1:10
High operative risk	2:10
Declined surgery	2:10

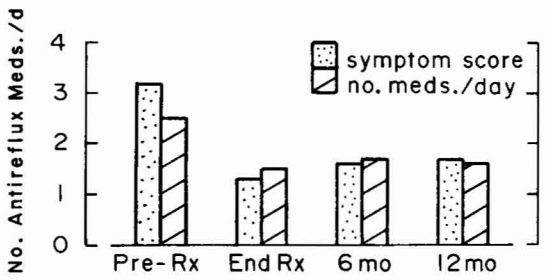

Figure 4. The mean symptom severity and number of antireflux medications taken per day before and after treatment.

score differs from the three posttreatment scores ($p < 0.001$), but the three posttreatment scores are not statistically different from one another. Before implantation the patients were taking an average of 2.5 different antireflux medications (8.2 doses) per day. At the conclusion of treatment they had spontaneously reduced their intake to a mean of 1.5 medicines (4.1 doses) per day. One year after treatment the mean number of medications per day was 1.6 (6.2 doses). Nine of the 10 patients reported symptom improvement after implant placement. Two patients had nearly complete relief of symptoms for about 10 months, 3 had definite symptom improvement for about 6 months, 3 were improved for 1 to 3 months, 1 patient with atypical chest pain had objective improvement in GER (but no change in symptoms), and 1 patient withdrew from the protocol.

The graded endoscopic appearances of the esophagus before, during, and after treatment are shown in Figure 5. One patient had a 3-cm Barrett's esophagus, 1 had undergone total gastrectomy for benign disease and had developed 10 cm of circumferential ulceration proximal to the esophagojejunostomy, 5 patients had linear erosions, 2 had linear erythema, and 1 had a highly irregular squamocolumnar junction with islands of proximal gastric epithelium. The mean (± SD) preinjection endoscopy score for all patients was 2.8 ± 1, at the completion of injections it was 1.9 ± 1, and 6 and 12 months after treatment it was 2.6 ± 1. As expected, there was no change in the length of metaplastic mucosa in the patient with Barrett's esophagus or in the patient with an irregular squamocolumnar junction and esophageal islets of gastric mucosa. The greatest visual difference in the mucosa occurred in the patient with an esophagus circumferentially ulcerated proximal to the esophagojejunal anastomosis. One year after treatment this patient still had two erosions but had extensive healing of the pretreatment ulceration. The endoscopic scores are not statistically different.

SART data depicting the number of reflux episodes before implant treatment, immediately after treatment, and 6 and 12 months after completion of treatment are shown in Figure 6. The mean (±SD) pretreatment number of reflux episodes was 8.2 ± 2, immediately posttreatment it was 3.0 ± 4 episodes, at 6 months it was 5.1 ± 5, and after 1 year it was 4.9 ± 5 episodes. There is a significant difference between pretreatment and all posttreatment SART scores ($p = 0.009$). The three posttreatment scores do not differ significantly from one another.

Treatment with injectable collagen resulted in increased LES manometric pressures (Fig. 7) in 8 of 9 patients (the LES was surgically absent in the patient who had had a total gastrectomy). With a mean pretreatment value of 15.7 ± 7 mm Hg (range, 4 to 24); the immediate postimplant mean LES pressure was 27.8 ± 6 mm Hg (range, 10 to 39); 6 months after treatment the mean LES pressure was 19.8 ± 6 mm Hg (range, 9 to 24); and 1 year after treatment it was 17.4 ± 7 mm Hg (range, 5 to 26). The immediately posttreatment LES pressure is significantly different from the three other pressure determinations ($p = 0.002$), but the pretreatment, 6-month, and 12-month pressures are not statistically different. All patients had normal LES relaxation with swallowing and normal peristalsis before and after implant injections.

Pretreatment esophagrams demonstrated reflux in 8 of 10 patients. After treatment 2 of 10 were positive; however, 6 months after collagen implantation, 5 of 9

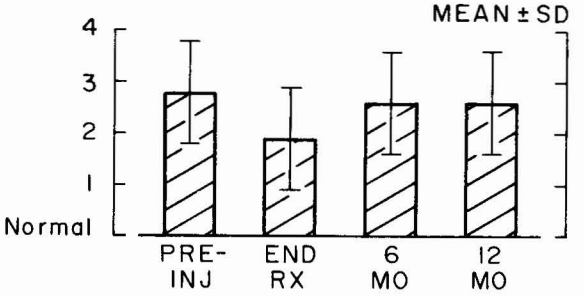

Figure 5. The mean endoscopy scores (0 to 4) before and after treatment.

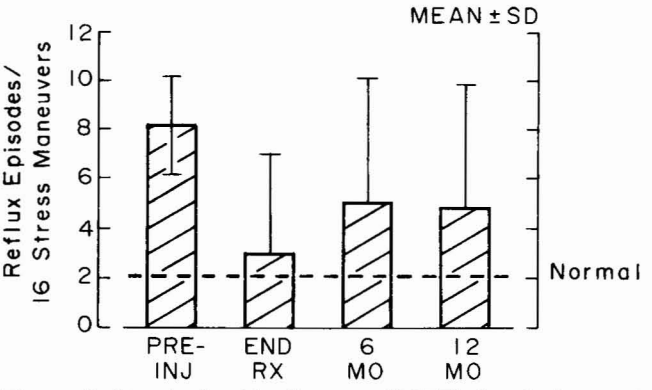

Figure 6. Standard acid reflux test (SART) data before and after treatment. The difference between the number of pretreatment and posttreatment reflux episodes is significant at the $p = 0.009$ level.

were again positive for GER, and 1 year later 5 of 7 esophagrams were positive for reflux (2 patients declined this test). The radiographic appearance of the distal esophagus was changed by the implants (Fig. 8). Esophagrams showed the caliber of the treated esophagus to be narrower and irregularly lumpy. The progress of a swallowed barium pill 13 mm in diameter was followed fluoroscopically in several patients. All pills passed normally through the implant zone, confirming the elasticity of the narrowed zone. Computed tomography scans were made through the distal esophagus with and without swallowed contrast in 2 patients. These scans showed the expected irregular thickening of the distal esophagus and the lack of any unusual bulk in the adjacent tissues or lymph nodes.

Nuclear scintiscans also showed postinjection improvement in reflux that tended to diminish over time. Before injections, 8 of 9 scans showed GER, after treatment 3 of 8 were positive, and 1 year after implantation 5 of 7 displayed reflux (2 patients declined this test).

No serious complications have been apparent; however, several minor side effects have occurred. Two patients had sufficient postimplant chest pain to require parenteral analgesics for 1 to 2 days (chest x-rays were unchanged). In 2 other patients small contact-point mucosal erosions were seen on implant mounds during follow-up endoscopy. Most patients experienced minor, transient dysphagia for solids for 1 to 3 days following implant injections. Serum antibodies developed to bovine (but not human) dermal collagen in 5 of the 10 patients. Patients with positive antibody titers have not developed clinically recognizable local reactions at either the implant or skin test sites. In addition, no other systemic symptoms were observed.

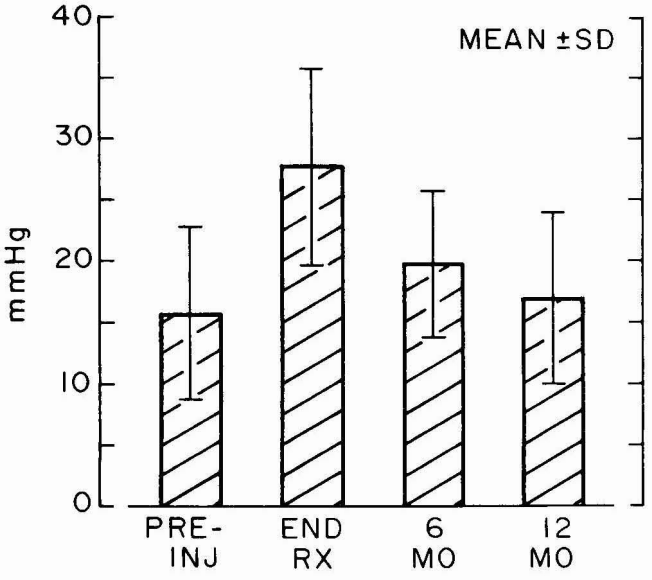

Figure 7. Mean LES pressures before and after treatment.

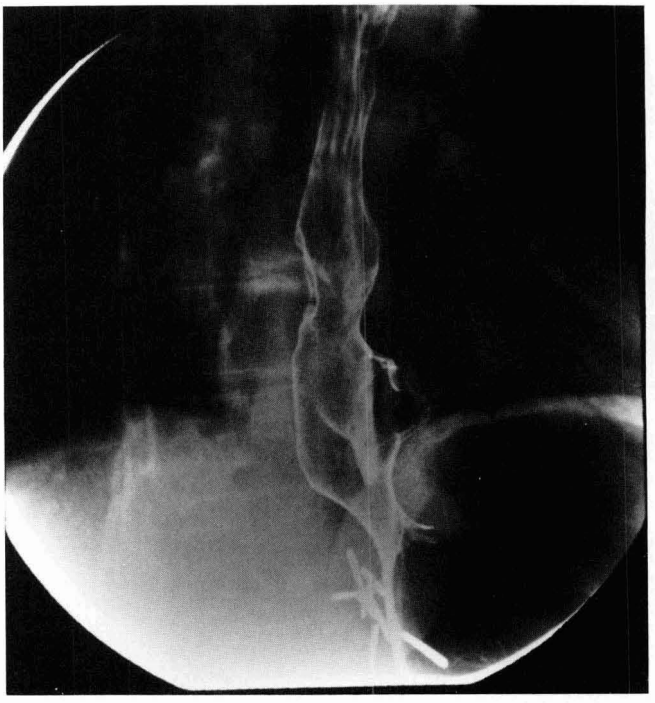

Figure 8. Double contrast esophagram showing that the implants appear as smooth overlapping mounds in the distal esophagus.

DISCUSSION

The management of GER patients who fail surgical therapy or fail medical treatment and are poor surgical candidates is frustrating. These patients with severe, intractable GER were therefore chosen as suitable subjects for experimental endoscopic implant treatment. Five of the patients had previously undergone a fundoplication, and 3 had had 2 antireflux operations. One patient had survived a cardiac arrest during induction of general anesthesia, 1 was morbidly obese, two had declined antireflux surgery, and another with an esophagojejunostomy had severe, ulcerating alkaline esophagitis. All were experiencing disabling, daily symptoms despite having taken up to 5 GER medications per day.

The initial clinical response to collagen implantation was favorable. Reflux symptoms decreased in all but 1 patient and were essentially absent in 3 patients. LES pressures increased in 9 of 10 patients by a mean of 12 mm Hg. The frequency of GER as assessed by nuclear scintiscan, esophagram, and number of medications taken per day decreased by one or more criteria in all patients. The improvement in GER as assessed by SART, symptoms, and LES pressure was statistically highly significant. It is possible that even better clinical results could have been achieved more readily if less severely afflicted patients had been treated. However, the limited precedent for this form of therapy and the extent of the unknown risk to the patient compelled us to accept only those patients who had

exhausted their nonsurgical therapeutic alternatives. The statistically significant changes in objective parameters (SART and LES pressure) make it highly unlikely that the results of treatment can be attributed to a placebo effect.

A mean of more than 6 injection sessions were used to deliver the implants. The 10 implant sessions that 2 of the early patients underwent are too many to be practical, but later patients received comparable implant volumes in 3 or 4 sessions. Larger injection volumes (per site and per session) were used when it became apparent that the risk of the collagen implant eroding through the mucosa was negligible. It is also probable that if a less refractory group of patients had been treated, a smaller volume of implant and fewer injection sessions would have been necessary to achieve therapeutic benefit.

The endoscopic injection of exogenous submucosal implants proved feasible, technically easy to perform, and well tolerated by patients. However, the major limitation of collagen implants is their lack of persistence. With time implant bulk became less apparent to the endoscopist, LES pressures decreased, and reflux symptoms recurred. When similar collagen preparations are injected into facial skin for their cosmetic effect, "touch up" reimplantation in 6 to 12 months is necessary to maintain the result.[5] Proteolytic degradation of collagen or the continued action of local stress and muscular activity presumably account for this effect.

Collagen has been successfully used in the biomedical devices for decades because of its properties of structural strength and biocompatibility.[6] To date, injectable bovine dermal collagen has been used in over 300,000 patients to treat soft tissue contour irregularities.[7] Cross-linking collagen grafts with glutaraldehyde enhances persistence and lessens hypersensitivity reactions.[8,9] Lightly cross-linked GAX collagen was utilized in these studies.

Five patients out of 10 developed antibodies to bovine collagen during this study. These antibodies demonstrated specificity for bovine collagen and did not cross-react with human collagen.[10] Antibody-positive patients did not develop symptoms that could be distinguished from the patient's reflux symptoms or the transient injection discomfort experienced by all patients, and no subject displayed any detectable systemic symptoms or had complaints. None of the patients with anticollagen antibodies developed any signs of inflammation at the skin test site that were evident to either the physician or the patient. One patient with an elevated titer of anticollagen antibodies reverted to a normal titer during the course of the clinical trial.

The presence of antibodies to bovine collagen in the absence of clinical manifestations of hypersensitivity has been recently described among patients treated with non cross-linked collagen for soft tissue contour irregularities.[11] However, the incidence of antibovine collagen antibodies in this study is far higher than that observed in patients treated with collagen for other indications.[10,11] This could be due to the volume of collagen employed in this study, to the visceral location of the implant, or the preexisting inflammation at the implant site.

Patients did not respond uniformly to esophageal implant therapy. Two factors with a possible bearing on the outcome of an individual's treatment was the development of antibody to the implant and whether the subject had undergone a prior fundoplication. Antibody-positive patients had a mean symptom-improvement interval of 4.3 months compared to 7 months for antibody-negative patients. LES pressures after implantation were similar in antibody-positive and antibody-negative patients (Fig. 9). Operated patients had a slightly higher preinjection LES pressure than unoperated patients (17.5 vs. 14.2 mm Hg), but the minor preinjection LES pressure differential does not seem sufficient to explain the discrepancy seen in how long the patient experienced symptom relief. While the immediate postinjection mean LES pressure for all patients was 27.8 ± 6 mm Hg, the mean pressure in unoperated subjects was 24 and the mean pressure in operated subjects was 32 mm Hg. The mean persistence of symptom relief was 7.3 months for operated patients and 3.8 months for unoperated patients. As 2 of the operated and 3 of the unoperated patients were antibody positive, there are too few patients to clearly distinguish between the effects of these 2 variables. However, it appears that a prior fundoplication confers a treatment benefit (Fig. 9). A speculative explanation for this is that the fundoplication wrap provides a thickened muscular zone in which to inject implant, giving more soft tissue support to the distal esophagus. The patient who achieved the longest asymptomatic posttreatment interval was antibody-negative and had had a fundoplication, but

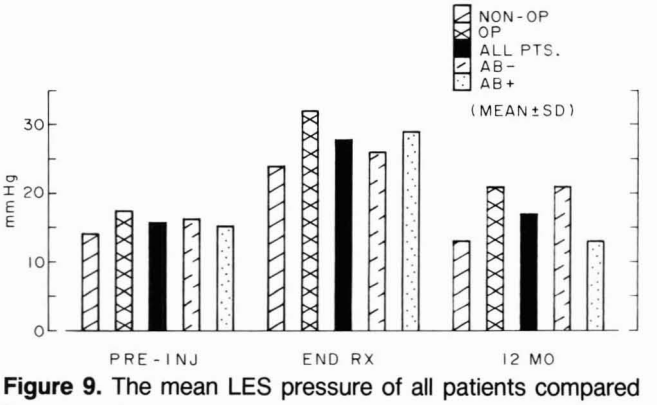

Figure 9. The mean LES pressure of all patients compared to unoperated versus operated patients and antibody-negative versus antibody-positive patients.

a close second was an antibody-positive, operated patient.

The theoretical advantages of LES implant therapy are several. The submucosal implants could restore and close the natural distal esophageal terminal rosette, while the uninjected tissue between the implants retained its distensibility and resilience. The combination of reinforced and elastic tissue should minimize the passive return of gastric contents into the esophagus while retaining the capacity to relax sufficiently to allow a swallowed bolus of food to pass through it. The exogenous support provided for the LES should complement the intrinsic neuromuscular apparatus.

The potential for this mode of treatment has been confirmed by this study. Specifically, the technique is well tolerated by outpatients and by patients considered to have an increased operative risk. Also, incremental treatment makes symptoms of overcorrection such as the gas-bloat syndrome, inability to vomit, and persistent dysphagia unlikely to occur. Finally, the LES pressure does increase with implant injection and both GER symptoms and objective GER test results show improvement with treatment.

What this study failed to accomplish was demonstration that the cross-linked bovine dermal implant was persistent. The immunogenicity of the implant was unforeseen because of the extensive prior testing done on the implant placed in the dermis.[7,8,11] It is speculated that the visceral location of the implant in patients with GER was responsible for the unprecedented rate of antibody formation, but the mechanism is not known. Another major goal of the study was to show that this mode of therapy is capable of producing sustained clinical benefit. For inoperable patients reinjection even at yearly intervals might be acceptable, but the mean duration of symptom relief for the implant employed in this study was too short for practical general use. Whether alternative implant materials (or refinement of this one) can provide sustained, biocompatible support for the LES is the subject of continuing study.

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REFERENCES

1. Ford CN, Martin DW, Warner TF. Injectable collagen in laryngeal rehabilitation. Laryngoscope 1984;94:513–8.
2. Politano VA. Periuretheral Teflon injection for urinary incontinence. Urol Clin N Am 1978;5:415–22.
3. Politano VA, Small MP, Harper JM, Lynne CM. Periurethral Teflon injection for urinary incontinence. J Urol 1974;111:180–3.
4. O'Connor KW, Madison SA, Smith DJ, Ransburg RC, Lehman GA. An experimental endoscopic technique for reversing gastroesophageal reflux in dogs by the injecting of inert material in the distal esophagus. Gastrointest Endosc 1984;30:275–80.
5. Klein AW, Rish D. Substances for soft tissue augmentation: collagen and silicone. Dermatol Surg Oncol 1985;11:337–9.
6. Chvapil M, Kronenthal RL, Van Winkle W. Medical and surgical applications of collagen. Int Rev Connect Tissue Res 1973;6:1–61.
7. Watson W, Kaye R, Klein A, Stegman S. Injectable collagens: a clinical overview. Cutis 1983;31:543–6.
8. Griffiths RW, Shakespeare PG. Human dermal collagen allografts: a three-year histological study. Br J Plast Surg 1982;35:519–23.
9. Carpentier A, Lemaigre G, Robert L, Carpentier S, Dubost C. Biological factors affecting long-term results of valvular heterografts. J Thorac Cardiovasc Surg 1969;58:467–83.
10. Ellingsworth LR, DeLustro F, Brennan JE, Sawamura S, McPherson J. The human immune response to reconstituted bovine collagen. J Immunol 1986;136:877–82.
11. McCoy JP, Schade WJ, Siegle RJ, Waldinger TP, Vanderveeh EE, Swanson NA. Characterization of the humoral immune response to bovine collagen implants. Arch Dermatol 1985;121:990–4.